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Exploration of ruthenium complex of (E)-2-((pyridine-2-yl) methyleneamino) benzoic acid as chemosensor for simultaneous recognition of acetate and HSO_4^- ions in cell bio-imaging: Experimental and theoretical studies

Jesús Alfredo Ortega Granados[a], Jose Guadalupe Hernández[b], Carlos Alberto Huerta-Aguilar[c], Pandiyan Thangarasu[a,*]

[a] Facultad de Química, Universidad Nacional Autónoma de México, Cd. Universitaria, 04510 México, D.F., México
[b] Centro Tecnológico, Facultad de Estudios Superiores (FES-Aragón), Universidad Nacional Autónoma de México (UNAM), Estado de México, CP 57130, México
[c] División de Ingeniería en Nanotecnología, Universidad Politécnica del Valle de México, Av. Mexiquense, Tultitlan, Estado de México, C.P. 54910, México

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ABSTRACT

A ruthenium complex $[RuL^1Cl_2(PPh_3)]Cl$ (L^1 = (E)-2-((pyridine-2-yl)methyleneamino)benzoic(*E*) acid) was synthesized, characterized by analytical techniques, and then it has been employed as chemo-sensor for the recognition of acetate in H_2O:CH_3CN (80:20) medium. With fluorescence study, it has been shown that $[RuL^1Cl_2(PPh_3)]^+$ efficiently detects acetate ion without interference from other anions (F^-, Cl^-, Br^-, I^-, NO_3^-, HSO_4^-, $H_2PO_4^-$) except HSO_4^-, for which the intensity of fluorescence is significantly enhanced. The stoichiometry determined by Job's plot for the interaction of Ru-complex with acetate was found to be 1:2. With DFT study, the energy of HOMO orbitals of the complex was calculated in order to address why the fluorescence intensity was enhanced for the addition of HSO_4^- ion, it establishes that the energy of HOMO for $[RuL^1(AcO)HSO_4]^-$ is being lowered when compared to that for $[RuL^1Cl_2(PPh_3)]^+$, inhibiting the excited electron transfer, and thus it increases the fluorescence intensity due to Photo induced Electron Transfer (PET). The recognition of acetate/or HSO_4^- has been successfully applied into real bio-sample such as *Saccharomyces cerevisiae* cells by performing confocal fluorescence with using the ruthenium complex.

1. Introduction

The detection of ionic or neutral species through receptor molecules has been considered as an interesting topic in analytical, biological and environmental sciences [1–6]. The binding of a receptor with a guest molecule generally provokes the whole unit (Host-Guest complex) to behave spectrally or electrochemically different from those of individual (receptor or guest) moiety. Thus several researchers intend to apply this concept to develop molecular-recognition/sensing systems for the detection of cation or anion, or neutral organic molecules in analytical/biological/environmental samples [7–12]. In this context, several luminescent metal complexes [13–16] or imine ligands [17–20] have been attracted as chemo-sensor for a series of analytes as they easily undergo a structural change during their binding with anion or neutral moieties; ultimately, it alters the metal-to-ligand charge transfer (MLCT) or ligand-to-metal charge transfer (LMCT), influencing spectral, redox, magnetic, and catalytic properties [21,22]. Hence, the luminescence probe for the recognition of anions becomes most popular in analytical techniques [23–25]. This is because ruthenium complexes [26–30] exhibit interesting photo-physical/electrochemical properties, playing a crucial role in recognizing anions (AcO^-, CN^-, F^- and $H_2PO_4^-$) [31–36]. In literature, the different anions have been selectively detected by using following complexes: $[Ru(bpy)_2L](ClO_4)_2$ (L = 2-(2-methoxy phenyl)-1H-imidazo[4,5-f][1,10] phenan-throline) [37], $[Ru(bpy)_2(H_3L)]^+$ (H_3L = 5-(1H-benzo [d]imidazol-2-yl)-1H-imidazole-4-carboxylic acid) [28], and $[Ru(bpy)_2(L)](PF_6)_2$ (L = calixarene) [38] for acetate, or/and F^- ions; ferrocene-imidazophenan-throline-based ruthenium(II)–polypyridyl complex[39] for chloride, $[Ru(bpy)_3](ClO_4)_2$ (bpy = 2,2′-bipyridine)[40] and

* Corresponding author.
E-mail address: pandiyan@unam.mx (P. Thangarasu).

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$[Ru(bpy)_2(mpipH)]$ $(ClO_4)_2$ (bpy = 2,2′-bipyridine; mpipH = 2-(4-methylphenyl)imidazo[4,5-f]-1,10-phenanthroline [40]) for CN^-.

It is known that the efficiency of molecular-recognition depends on many factors, of which solvent medium plays a vital role for the formation of hydrogen bonds between host molecule and guest moiety [41–44]; of course, the type of ligand attached to the binding site, and the pH of the medium determine the nature of the H-bonds [45–47] that influence to develop a signal depending on the variation of the size and shape of host or guest moieties [48–50]. The whole issue depends mostly on hydrophobic or hydrophilic nature in polar solvents [51,52]. Additionally, the type of guest ions such as atomic radius, hardness/softness, the charge of ion, and type of orbital involved in the bond formation are also to be considered in developing molecular-sensor system [53,54].

Previously, we used ruthenium complexes derived from N,N'-bis(salicylidene)ethylene diamine (L^1) [55] or from benimidazole based ligands [8] for the recognition of anions (acetate and chloride). With our continuing efforts, we synthesized a ruthenium complex $[RuL^1Cl_2(PPh_3)]^+$ (L^1 = (E)-2-((pyridine-2-yl)methyleneamino)benzoicacid) that recognizes acetate/HSO_4^- ions in bio-samples. The detection of anions is essential as it can be applied in many chemical, biological, and environmental samples [56–67] beside several co-factors and other biochemically relevant substrates are in anionic nature. In particular, acetate ions involve considerably in various metabolic functions through hydrogen bond formation (enzyme activity and antibody functions) [68–71]. In the present study, it has been found $[RuL^1Cl_2(PPh_3)]^+$ recognizes selectively acetate ions by quenching of the fluorescence intensity without interference from other ions (F^-, Cl^-, Br^-, I^-, NO_3^-, $H_2PO_4^-$) except HSO_4^-. Furthermore, with using DFT studies, we illustrate here why the fluorescence property of the complex is being altered during the binding of acetate or HSO_4^-. Finally, with Confocal microscopy analysis, the cell bio-images are developed by interacting the Ru complex with acetate or HSO_4^- in the *Saccharomyces cerevisiae* cells.

2. Experimental

2.1. General annotations

All chemicals (analytical grade) were used as received from Sigma-Aldrich without any further purification. The percentage of elemental content (C, H, N) was determined on an elemental analyzer (Fisons instrument, model EA 1108 CHNSO). With a NMR equipment (Varian Gemini, 300 MHz), ^1H, and ^{13}C NMR spectra were recorded for the compounds and TMS was used as internal standards. The mass spectral fragments of the compounds were determined by a GC–MS (Joel JMS-Axsosha instrument). UV–vis absorption spectra on a spectrophotometer (Perkin Elmer, Lambda 25), and fluorescence spectra on a F96 Pro spectrophotometer were recorded.

2.2. Synthesis of the ligand (L^1)

Ligand L^1 was synthesized as reported elsewhere [72–75]. Anthranilic acid (1.37 g, 10.0 mM) dissolved in MeOH (25.0 mL) was added drop-wise to 2-pyridyl carboxylaldehyde (1.07 g, 10.0 mM) dissolved in MeOH (25 mL). The resulting mixture was stirred at room temperature for 2 h before the solvent was evaporated by a roto-evaporator, yielding a yellow-orange semi-solid and it was purified by column chromatography using mobile phase $MeOH/CH_2Cl_2$ (1:4), and re-crystallized from $MeOH/CH_2Cl_2$ (1:5). The yellow solid formed was filtered and washed several times with diethyl ether. Yield: 1.72 g, 75%. $C_{13}H_{10}N_2O_2$: C 69.02%, H 4.46%, N 12.38%; observed: 69.13%, H 4.42%, N 12.18%. ^1H NMR (300 MHz, CD_3OD): (COOH, s, 11.05), N=CH (2H, s, 8.55), (4H, aromatic, m, 6.65–8.75). M/z: $C_7H_5O_2$ (100%), $C_{12}H_{10}N_2$ (183.2), $C_{13}H_9N_2O_2$ (227.2 M^+).

2.3. Synthesis of $[RuL^1Cl_2PPh_3)]Cl$

Ruthenium complex with ligand L^1 was prepared as indicated in Ref. [76]; typically, to a methanolic solution of L^1 (0.11 g, 0.5 mM, 20 mL), $[RuCl_2(PPh_3)_3]$ (0.47 g, 0.5 mM) was added, and the resulting solution was refluxed for 12 h before cooling it to room temperature. The solvent was removed by the roto-evaporation to yield a residue, which was then washed with diethyl ether. A dark brown product obtained was re-crystallized from methanol: Yield (0.44 g, 23%). Elemental analysis: calcd. $C_{31}H_{25}N_2O_2Cl_3PRu$: C 53.59%, H 3.63%, N 4.03%; observed: C 53.51%, H 3.83%, N 3.94%. ^1H NMR (300 MHz, CD_3OD): N=CH (H, s, 8.30-8.50), H_{arom} (8H, m, 7.10–8.0); ^{13}C NMR (300, MHz, CD_3OD): N=CH (157.50), phenyl rings and PPh_3 (125.0 −138.0).

2.4. Anion recognition studies by $[RuL^1Cl_2(PPh_3)]^+$

The ruthenium complex was tested as chemosensor for the recognition of anions with employing the following studies: (i) binding tests, (ii) competitive binding (interference) studies, and (iii) titration analysis. All the experiments were carried out in triplicate at room temperature. In an anion binding analysis, typically, to a standard solution of $[RuL^1Cl_2(PPh_3)]^+$ (3.0 mL, 0.1 mM) dissolved in H_2O:CH_3CN (80:20%, *v*/*v*), a fixed amount of different anion in the form of tetrabutylammonium (TBA) salt (0.1 mM) was first added, and stirred for the homogeneity before recording the fluorescence spectra, and observed if $[RuL^1Cl_2(PPh_3)]^+$ changes the characteristic of the fluorescence behavior significantly. The results show that the complex selectively detects acetate ion after testing following anions: F^-, acetate, Cl^-, Br^-, I^-, NO_3^-, HSO_4^-, $H_2PO_4^-$. In the competitive binding analysis, we used other coexistent ion in a competitive medium for the detection of acetate, and observed if there is any interference from other coexistent anion that alters the fluorescence intensity generated by $[RuL^1Cl_2(PPh_3)]^+$ with acetate ion. This means, the sensitivity of $[RuL^1Cl_2(PPh_3)]^+$ (0.1 mmol) was analyzed as receptor for the binding of acetate ion (0.1 mM) in a competitive medium having additional anion. In the titration analysis, usually, to $[RuL^1Cl_2(PPh_3)]^+$ (2.0 mL, 0.1 mmol), tetrabutylammonium acetate (10 μL, 0.0–0.025 M) was added, and for each subsequent addition, the fluorescence intensity measured was plotted against the concentration of acetate. The stoichiometry (binding ratio) for the interaction of the complex with acetate was determined by Job's method [77,78]. To a set of solutions having a constant total concentration of the receptor $[RuL^1Cl_2(PPh_3)]^+$ and the anion were prepared. For the complex, the plot corresponding to the concentration at which the fluorescence intensity to be zero was obtained. The intensity of the complex was quenched for the successive addition of acetate, and it was increased for HSO_4^- addition to the mixture of [acetate + Ru complex].

2.5. Bio-imaging studies

The fluorescence bio-image for the recognition of acetate or HSO_4^- by using the ruthenium complex in *Saccharomyces cerevisiae* cells was studied. In particular, with the cells, the fluorescence intensity of the complex in the presence and the absence of acetate (450–500 nm) was monitored in an Olympus FV1000 instrument, equipped with a diode laser (405 nm) as excitation source; in the same way, the fluorescence images were obtained for [complex + acetate] + with HSO_4^-. These cell culture experiments were